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| **DECLARATION OF JAMES B TRAGER UNDER 37 C.F.R. § 1.132** | Attorney Docket | UCAL-269 |
| | First Named Inventor | P. Choudary |
| | Confirmation Number | 9044 |
| | Application Number | 09/425,075 |
| | Filing Date | October 21, 1999 |
| | Group Art Unit | 1642 |
| | Examiner Name | Larry Helms |
| | Title: *FUNCTIONALLY ASSEMBLED ANTIGEN-SPECIFIC INTACT RECOMBINANT ANTIBODY AND A METHOD FOR PRODUCTION THEREOF* | |

Dear Sir:

1. I, James B. Trager, declare and say I am a resident of the State of California. My residence address is 1308 Park Avenue, Alameda, California.

2. I hold a B.A. degree in Philosophy, which I received from St. John's College, Santa Fe, in 1984. I further hold an Ph.D. degree, which I received from the University of California at Berkeley, in 1994. I currently work as a Senior Scientist at Geron Corporation.

3. I did my Ph.D. in the field of gene expression in *Saccharomyces cerevisiae*, and, during the course of my research career, I have worked with several different species of yeast, including *Saccharomyces cerevisiae* and *Pichia pastoris*, sometimes for expression of heterologous proteins. Details of my career and publications may be found in my *curriculum vitae*, provided herewith. I am therefore very familiar with yeast in general, and am well qualified to offer my opinion on what a researcher of ordinary skill in the art would consider obvious in the area of antibody expression in *Pichia* in October 1999, the filing date of this application.

4. I have reviewed the claims, and understand that the claimed invention (i.e., the "Invention" is a method for expressing antibodies in *Pichia* using a dual expression cassette vector, and vectors and host cells for performing the method. I have also reviewed the publications (i.e., the "cited publications") of Robinson et al., Horwitz *et al*, Cregg *et al*, The Invitrogen Catalog 1997, and Sambrook *et al*, as cited in the rejection set forth in the Office Action dated April 16, 2003.

5. I have been asked to opine of the following general question:

In view of the cited publications, would one of skill in the art, in October 1999, think that a method for expressing antibodies in *Pichia* using a dual expression cassette vector is obvious?

It is my unequivocal opinion, based on the facts and reasoning set forth below, that the answer to this question is "no".

6. It is my understanding that the cited publications are to be viewed from the standpoint of one of ordinary skill in the art in the relevant field (a "Skilled Person") at the time of filing of the patent application in question. The patent application in question was filed on October 21, 1999, and relates to the field of heterologous protein expression in yeast. I would expect a Skilled Person in the field of heterologous protein expression in yeast, immediately prior to and up to October, 1999 (the "relevant period") to have been represented by a scientist with a Ph.D. degree. I consider that such a Skilled Person would have the ability to make constructs for expressing heterologous proteins in yeast without inventive effort.

7. Since during the relevant period I a) was a Skilled Person and b) regularly attended external and internal meetings at which Skilled Persons presented their

research, I believe that I am qualified by training and experience to address what a Skilled Person would have understood from a reading of the cited publications.

8. There are three main bases for my opinion that a Skilled Person would not conclude that the Invention is obvious in view of the cited publications. I will detail those reasons below:

The cited references, independently or together, do not suggest using a dual expression cassette vector for use in *Pichia*

9. The cited references, independently or together, do not suggest using a dual expression cassette vector for use in *Pichia*. The reasoning behind this statement is set forth below:

10. Robinson is the only reference that discusses dual-expression cassette vectors, and a suggestion to use such vector in "yeast" may be found in column 16 of Robinson. A Skilled Person would not equate "yeast" with "*Pichia*" in Robinson, and, as such, a Skilled Person would find no suggestion to use dual expression cassette vectors for antibody production in *Pichia*.

11. As is known by the Skilled Person, the word "yeast" has one of two meanings, depending on the context of how it is used. In the first meaning, "yeast" solely refers to the species of *Saccharomyces cerevisiae*, commonly known as "brewer's yeast". For example, if a Skilled Person says he works in a "yeast lab", he is indicating that he works in a lab that works on *S. cerevisiae*. In the second meaning, "yeast" refers to a genus of fungi that encompasses over 25,000 species from the following families *Saccharomyes, Pichia, Candida, Schizosaccharomyces, Neurospora*, and others. As an example, throughout this declaration I have used the word "yeast" in its second meaning, referring to a genus of fungi. In other words, depending on the context of

how the word "yeast" is used in a reference, it refers to either *S. cerevisiae*, or a genus of over 25,000 species of fungi.

12. From the context of how the word "yeast" is used in Robinson, a Skilled Person would recognize that Robinson uses the word yeast with its first meaning, as a reference to *S. cerevisiae*. A Skilled Person would recognize this because Robinson uses the terms, "yeast" and "*S. cerevisiae*" interchangeably. For example, Robinson refers to the *S. cerevisiae* gene as "the yeast invertase gene", refers to the *S. cerevisiae* PGK promoter as "the yeast PGK promoter", and refers to the origin of replication of the 2-micron plasmid endogenous to *S. cerevisiae* as "the yeast origin of replication, oriY, a cis-acting sequence (REP3) from the yeast endogenous 2-micron plasmid." At no point in the disclosure does Robinson suggest that "yeast" encompasses anything other than *S. cerevisiae*.

13. Upon reading the Robinson reference as a whole, a Skilled Person would recognize that the "yeast" referred to by Robinson is, in fact, *S. cerevisiae*, not a genus of fungi. Any suggestion by Robinson to use a dual expression cassette to express an antibody in yeast, is, therefore, a suggestion to use a dual expression cassette to express an antibody in *S. cerevisiae*. Since *S. cerevisiae* and *Pichia* are different species, a Skilled Person would find no suggestion in Robinson to use dual expression cassette vectors for antibody production in *Pichia*.

14. Based on the reasoning set forth above, it is my unequivocal opinion that a Skilled Person would find no suggestion in Robinson to use a dual expression cassette vector for antibody production in *Pichia*. Since this suggestion is not provided by any of the other cited references, the cited references, independently or together, do not suggest using a dual expression cassette vector for use in *Pichia*.

Successful heterologous protein expression in *S. cerevisiae* does not predict successful heterologous protein expression in *Pichia*

15. *S. cerevisiae* and *Pichia* are very different, phylogenetically distinct, species. It follows that a Skilled Person would recognize that even if a protein could be expressed in one species, there would be no reasonable expectation of success that it could be expressed in the other. For example, even if functional antibodies were shown to be expressed in *S. cerevisiae*, a Skilled Person would have no reasonable expectation of success in expressing the same antibodies in *Pichia*.

16. As such, a Skilled Person would recognize that an example of expressing a heterologous protein, such as an antibody, in *S. cerevisiae* would have no bearing whatsoever on whether or not that same heterologous protein could be expressed in *Pichia*. Even if a reference was cited that actually showed a working method for the expression of functional antibodies in *S. cerevisiae* using a dual expression cassette vector, it is my unequivocal opinion that a Skilled Person would have no reasonable expectation of success in practicing such a method in *Pichia*.

The art would lead a Skilled Person from combining the cited references

17. The Invention involves a dual expression cassette vector for expression of immunoglobulin heavy and light chains of an antibody. Because two expression cassettes are on the same vector, a Skilled Person, would not have any reasonable expectation of success in making and using such a vector because of the problems associated with intra-molecular recombination (*e.g.* occurring when two parts of a vector are similar in nucleotide sequence), transcriptional interference (*e.g.* occurring when transcription of one expression cassette does not terminate properly, so that transcription "reads through" so as to interfere with transcription of the second expression cassette), and translational interference (*e.g.* occurring when

transcriptional read-through of the first expression cassette produces an antisense molecule that interferes with the translation of the RNA from the second expression cassette). Such problems are commonly associated with such dual expression cassette vectors, especially when the expression cassettes contain polynucleotides with similar or identical sequences (for example similar promoters, signal sequence-encoding polynucleotides or terminators). The usual way of making vectors usually involves an intermediate vector production step in bacteria (*e.g.*, *E. coli*) these problems would pose serious technical barriers. Thus, even if these problems only happened in bacteria, they would still impact the question of whether or not a Skilled Person would make and use such a vector.

18. The following publications support this position: Hoshizaki (*Mol Cell Bio*, 1985 5:3323-9), Peterson (*J. Bact.*, 1983 156: 177-85); Nies (*J. Antimicrob Chemother.* 1986 18:Suppl 35-41); and a page of technical material found at Stratagene's website (http://www.stratagene.com/displayProduct.asp?productId=290). I am told that these publications have been previously provided to the Examiner.

19. Further, with specific reference to antibody expression in *Pichia*, there appears to be a significant amount of scientific literature that would lead a Skilled Person away from combining the cited references. For example, two reviews of the scientific literature on antibody expression in *Pichia* each discourage the Skilled Person from the expression of antibodies in *Pichia* using dual expression cassette vectors.

20. The first of these references, Pennell (*Res Immunol.* 1998 149:599-603; Exhibit A), states "The size of the protein to be expressed may also be limiting because to our knowledge, there are no reports of proteins greater than 117 kDa being expressed in *P. pastoris*." Since antibodies are generally larger than 117 kDa, Pennell's disclosure would lead a Skilled Person away from expressing a whole antibody in *Pichia*.

21. The second of these references, Holliger (*Methods in Mol Biol.* 2002 178:348-357; Exhibit B), states, in point 8 on page 351 "Because bicistronic expression works only poorly in *Pichia* (unlike *E. coli*), it is preferable to use single-chain Ab formats. Two chain Ab formats require that the two chains be cloned and transformed separately". (Underlining added). Hollinger, therefore, appears to say that single expression cassette vectors are **required** if expression of two different chains of an antibody is desired.

22. Based on the foregoing discussion, it is my unequivocal opinion that a Skilled Person, in view of the cited publications (i.e., Robinson et al, *etc.*), would not find the invention obvious because the literature and common knowledge in the field would lead them away from doing so. Given that two different reviews of the field of antibody expression in *Pichia* categorically and in no uncertainty direct away from using dual expression cassette vectors, why would a Skilled Person expect it would work?

23. In summary, and in view of the technical problems and guidance set out in the scientific literature as exemplified by the reviews discussed above, it is my unequivocal opinion that a Skilled Person would not conclude that the Invention is obvious in view of the cited publications. A Skilled Person would find no specific motivation to combine the cited publications to provide the Invention, and, in fact would be strongly led away from the invention.

24. I acknowledge I have been paid $250 for my services in reviewing the materials described herein, and in rendering this opinion.

25. I, James B. Trager, hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge

that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

July 9, 2003
Date

James B. Trager, Ph.D.

Attachments:

Exhibit A: Holliger, *Methods in Mol Biol.* 2002 178:348-357
Exhibit B: Pennell, *Res Immunol.* 1998 149:599-603

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